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Radiologic Tumor Measurements to Quantify Response to
Neoadjuvant Chemotherapy

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13. ABSTRACT (Maximum 200 Words) The aim of the first year of this project was to develop a new method to quantify the proportion (percent) of cancer that is residual after neoadjuvant chemotherapy using standard radiologic and/or clinical measures of tumor size integrated with pathologic information about the amount of cancer within each tumor. We tested tumor cellularity and combined that information with tumor size. We have determined that tumor cellularity significantly decreases as a result of neoadjuvant (pre-operative) chemotherapy compared to control untreated breast cancers managed by surgery alone. However, the extent and variability of reduction of cellularity is considerable, particularly in the partial and minimal clinically responsive groups. When cellularity is combined with actual tumor size, there is a shift in the distribution of this measure of residual cancer in the tumor bed, suggesting that most breast cancers are more responsive to neoadjuvant chemotherapy than measurement of tumor diameter alone would indicate. Therefore, size alone is not sufficient measure of the tumor response to treatment. We are now working to incorporate our measure of cancer cellularity to combine with the radiological tumor measurements for these patients during the course of their therapy to test the product of tumor size and cellularity as a measure of actual breast cancer response.				
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INTRODUCTION:

A more accurate way to measure breast cancer response to treatment would improve the rate of yield of information from clinical trials of neoadjuvant chemotherapy. It would also provide a more useful standard with which to compare the relevance of pathologic findings in residual cancer and with which to test those molecular biomarkers that show promise to predict response to treatment. We intend to develop and validate a method to quantify tumor response, using clinical, radiologic, and pathologic information that is applicable to most clinical practices. We will study the pathologic changes in the residual carcinoma from neoadjuvant chemotherapy as they relate to assessment of tumor response and molecular evidence of cell survival and proliferative activity in the residual cancer cells.

BODY:

Task 1. To determine the best measurement of tumor size after treatment (Months 1 - 24)

- a. Review of mammography and ultrasound imaging studies from before and after treatment, estimate average of 10 cases per month. (Months 1 - 24)*
- b. Two radiologists to independently make measurements and document the preferred imaging modality for each tumor. (Months 1 - 24)*
- c. Obtain the clinical tumor measurements and the categorical assessments of tumor response from the clinical trial database. (Months 1 - 6)*
- d. Pathology review of slides, reports, and specimen radiographs to document residual tumor size and other histopathologic findings for subsequent tasks. (Months 1 - 24)*
- e. Complete the statistical analyses. (Months 24 - 25)*

The Department of Defense approved the IRB for human subjects research on December 22, 2002. In seven months since then we have identified a cohort of 108 patients who received neoadjuvant chemotherapy for breast cancer and whose pathology slides have been retrieved from the files and reviewed (see task 2). Pathological data have been annotated for these 108 breast cancers, both before and after neoadjuvant chemotherapy. Those data include: tumor size, % invasive cancer, % in situ cancer, % cancer cellularity within the tumor, and cytomorphologic changes within residual cancer cells. A radiology research assistant has been recruited and has retrieved the films from the first group (20) of those patients. A data collection template was designed and formatted by the study radiologist (Dr. Whitman) in conjunction with the PI and the biostatistician (Dr. Smith). The radiologic measurement data are not yet ready for analysis.

Task 2. Calculation of percent residual cancer volume (Months 1 - 27)

- a. *Immunohistochemical staining of tumor sections for cytokeratins. (Months 1 - 24)*
- b. *Image analysis to calculate percent cancer cellularity by area. (Months 3 - 24)*
- c. *Calculation of tumor volume using the best measure of tumor size - see task 1. (Months 24 - 26)*
- d. *Calculation of percent residual cancer volume and statistical analyses. (Months 25 - 27)*

Cancer cellularity within the tumor area has been measured from hematoxylin and eosin stained tumor sections for the pre-treatment diagnostic core biopsy and the post-treatment resection specimen from 108 breast cancers from women who were treated with pre-operative (neoadjuvant) chemotherapy in the clinical trial (ID98-240). These were compared to the cancer cellularity in the diagnostic core biopsy and the surgical resection specimen from a control group of 120 breast cancers that did not receive pre-operative chemotherapy. Immunohistochemical staining for cytokeratin is underway, but those stains are not complete or ready yet for analysis.

Summary of the tumor cellularity in treatment and control groups

The tumor cellularity in treatment and control groups is summarized in Figure 1 using a boxplot. The black rectangle in each case indicates the 25th and 75th percentiles of the distribution with median indicated by white horizontal lines within the rectangles. The figure indicates that there was a significant overall decrease in cellularity of resection specimens compared to biopsy specimens for the treatment group (Paired Wilcoxon signed rank test p-value <0.01), while a significant increasing trend was noted in the percentage of tumor cellularity in patients from the control group (Paired Wilcoxon signed rank test p-value < 0.01).

Change in tumor cellularity in treatment and control groups

The relative changes in cellularity between biopsy and resection specimens are summarized in Figure 1(C) for the control and treatment groups. Relative change in tumor cellularity = (percentage of tumor cellularity at resection - percentage of tumor cellularity in biopsy) / percentage of tumor cellularity in biopsy. Medians (range) of the change in tumor cellularity in treatment and control groups were -0.67 (-1, 2.6) and 0 (-0.75, 5), respectively. (Note that values below 0 indicate lower cellularity at resection, e.g., a change from 60% cellularity to 30% cellularity would correspond to a value of -0.50). P-value from Wilcoxon rank sum test was less than 0.01. It is apparent from Figure 1(C) that there was a major trend for decreasing tumor cellularity after patients received neoadjuvant chemotherapy.

Association of change in tumor cellularity with clinical response and stage (n=108)

Among 108 patients receiving neoadjuvant chemotherapy, 31(29%) patients achieved clinical complete remission (CR), while 62(57%) patients achieved clinical partial complete remission. The association of change in tumor cellularity with clinical response is illustrated in Figure 2 (a). It indicates that patients who achieved CR had significantly

larger reduction in tumor cellularity than other patients (Kruskal-Wallis test p-value <0.01, p-value is also less than 0.01 after combining minimal response and progressive disease). Figure 2 (b) shows that there was a major trend for increasing change in cellularity as tumor size increased (Kruskal-Wallis test p-value <0.01). Cellularity in patients with small tumors was much lower after treatment than before treatment. A T0 tumor had zero cellularity by definition. (P-value from Kruskal-Wallis test is less than 0.01 after T0 is excluded).

Clinical response by treatment schedule

Patients in the neoadjuvant group all received the same chemotherapy agents but administered by different schedules, either weekly or every 3 weeks. Response results are summarized in the following table:

Table 1 Distribution of clinical response by treatment

Schedule	Clinical response			
	CR	PR	MR	PD
1-week	19(34%)	29(52%)	7(12%)	1(2%)
3-week	12(23%)	33(63%)	5(10%)	2(4%)

We have confirmed that the association between change in cellularity and clinical response was similar on the two treatment schedules (Figure 3) (p-values from Kruskal-Wallis test are less than 0.01 for both schedules after combining minimal response and progressive disease).

A summary of the results from above was prepared and submitted in abstract form for consideration for the San Antonio Breast Cancer Symposium that is scheduled for December, 2003.

Combining pathologic tumor size with cancer cellularity

The product of tumor diameter from the resection specimen and the cancer cellularity within the resected tumor was compared with tumor diameter alone in the treated and control groups (Figure 4). Frequency distributions for tumor size alone are similar in the treated and control tumors (Figure 4a, 4b). The product of size and cellularity has a similarly skewed normal distribution in the control tumors (Figure 4c), but in the treated tumors the distribution appears to be different (Figure 4d). The shape of that distribution (Figure 4d) suggests that the population of treated tumors all tend towards a zero product of size and cellularity (complete response) rather than having a skewed normal or bimodal distribution. This appears to be a meaningful distribution because it suggests that almost all tumors respond to treatment to some extent. The implication of these graphs is that the incorporation of cancer cellularity as a variable in the measurement of tumor response is likely to be an improvement over size alone. Thorough statistical analysis of these data is currently being performed. When that is complete the results from the above analyses will be submitted as a manuscript for publication in a journal.

Task 3. To assess the pathology of residual cancers and correlate these with tumor response. (Months 12 - 30)

- a. Immunohistochemical staining of residual tumor sections for Ki-67/MIB-1, HIF-1 α , bcl-2, bcl-XL, and NF- κ B. (Months 12 - 20)*
- b. TUNEL assay for apoptosis in residual tumor sections. (Months 20 - 24)*
- c. Microscopic interpretation of immunohistochemistry and TUNEL staining. (Months 20 - 28)*
- d. Complete the statistical analyses with tumor response. (Months 28 - 30)*

Work on Task 3 is scheduled to begin in year 2 of funding.

Task 4. To test selected potential biomarkers for prediction of tumor response. (Months 24 - 34)

- a. Immunohistochemical staining of pre-treatment tumor samples for Ki-67/MIB-1 and p53. (Months 24 - 30)*
- b. Retrieval of results from Her-2/neu tests from pathology reports. (Months 24 - 27)*
- c. Microscopic interpretation of immunohistochemical staining and histopathologic biomarkers. (Months 28 - 32)*
- d. Complete the statistical analyses with tumor response. (Months 32 - 34)*

Work on Task 4 is scheduled to begin in year 3 of funding.

Task 5. Compilation of patient follow-up from clinical trial database and statistical analyses for disease free interval and survival. (Months 30 - 36)

Work on Task 5 is scheduled to begin in year 3 of funding.

KEY RESEARCH ACCOMPLISHMENTS:

Key research accomplishments from the first period of study are:

- Demonstration that cancer cellularity within the tumor is significantly decreased by neoadjuvant chemotherapy,
- Reduction in cancer cellularity is most obvious and variable in the partial response and minimal response (stable disease) categories and, similarly, in tumors staged as T1 after treatment,
- Distribution of the product of tumor size and cancer cellularity after treatment demonstrates more clearly than the tumor size alone that almost all tumors achieve a response from neoadjuvant chemotherapy,
- This distribution of the product of size and cellularity appears likely to be biologically meaningful and might be amenable to mathematical modeling,
- Combining size with cellularity is likely to improve the accuracy of tumor response measurement.

REPORTABLE OUTCOMES:

Abstract submitted to the San Antonio Breast Cancer Symposium for December, 2003.
Rajan R, Poniecka A, Smith T, Yang Y, Whitman G, Fiterman DJ, Pusztai L, Kuerer H, Hortobagyi GN, Symmans WF. Tumor cellularity of breast cancer as a variable in the pathological assessment of response following neoadjuvant chemotherapy.

CONCLUSIONS:

1. Assessment of cancer cellularity within the measured tumor bed provides meaningful information about tumor response following therapy.
2. Planned future studies of this variable with radiologic tumor measurements (before and after treatment) from this clinical trial are likely to yield valuable results.
3. Refinement of the assessment of cancer cellularity using cytokeratin immunohistochemical stains will be studied.

REFERENCES:

None

APPENDIX:

Figures 1 - 4

FIGURE LEGENDS:

Figure 1. Box plots to show the means and standard deviations of the cancer cellularity assessed from tumor tissue sections from the core biopsy and subsequent resection specimen in 108 patients who received neoadjuvant chemotherapy (A) and 120 control patients who did not receive neoadjuvant chemotherapy (B). The relative change in cancer cellularity is presented for all patients in (C). Neoadjuvant chemotherapy reduces cancer cellularity in the tumor bed.

Figure 2. There is reduced cancer cellularity in clinically partially and minimally responsive tumors, but that change in cellularity is highly variable in those clinical response categories (A). Change in cancer cellularity was compared to final pathological tumor stage (B). There was most reduction in cancer cellularity in the T1a and T1b tumors, but that was also most variable.

Figure 3. Comparing the two treatment arms in the clinical trial, there was a difference in the partial responders, with greater relative reduction in cancer cellularity in the weekly paclitaxel group (A) than in the 3-weekly paclitaxel group (B). So not only was there a higher complete pathologic response rate in the weekly paclitaxel group, but also the clinical partial responders in that group had more reduction of cancer cellularity in the tumor bed following treatment.

Figure 4. Pathologic tumor diameter is compared in 120 untreated control breast cancers (A) and 108 treated breast cancers (B). The distributions of sizes are similar in the two populations, except for a group of complete pathological responses after treatment (tumor size = 0). When the product of pathologic tumor diameter and cancer cellularity was calculated, the control cancers retain a similar distribution (C), but the distribution of the treated tumors is different (D). The distribution in the treated cancers resembles an inverse logarithmic curve and suggests that the response to chemotherapy in the majority of cases is greater than size alone would predict. Incorporation of cancer cellularity as a variable appears to organize the tumor measurements into a more biologically likely distribution that tends towards complete remission.

Figure 1

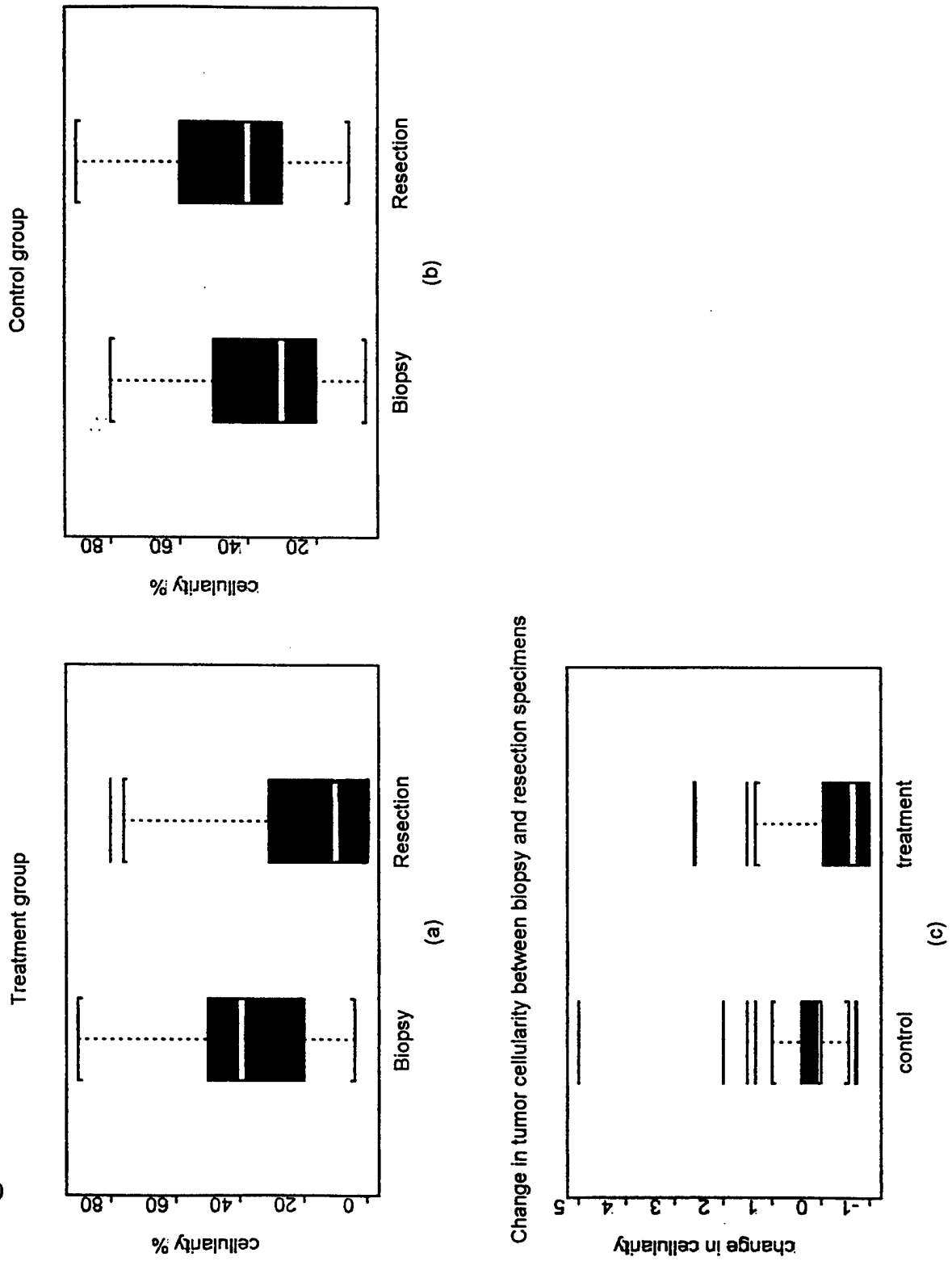


Figure 2

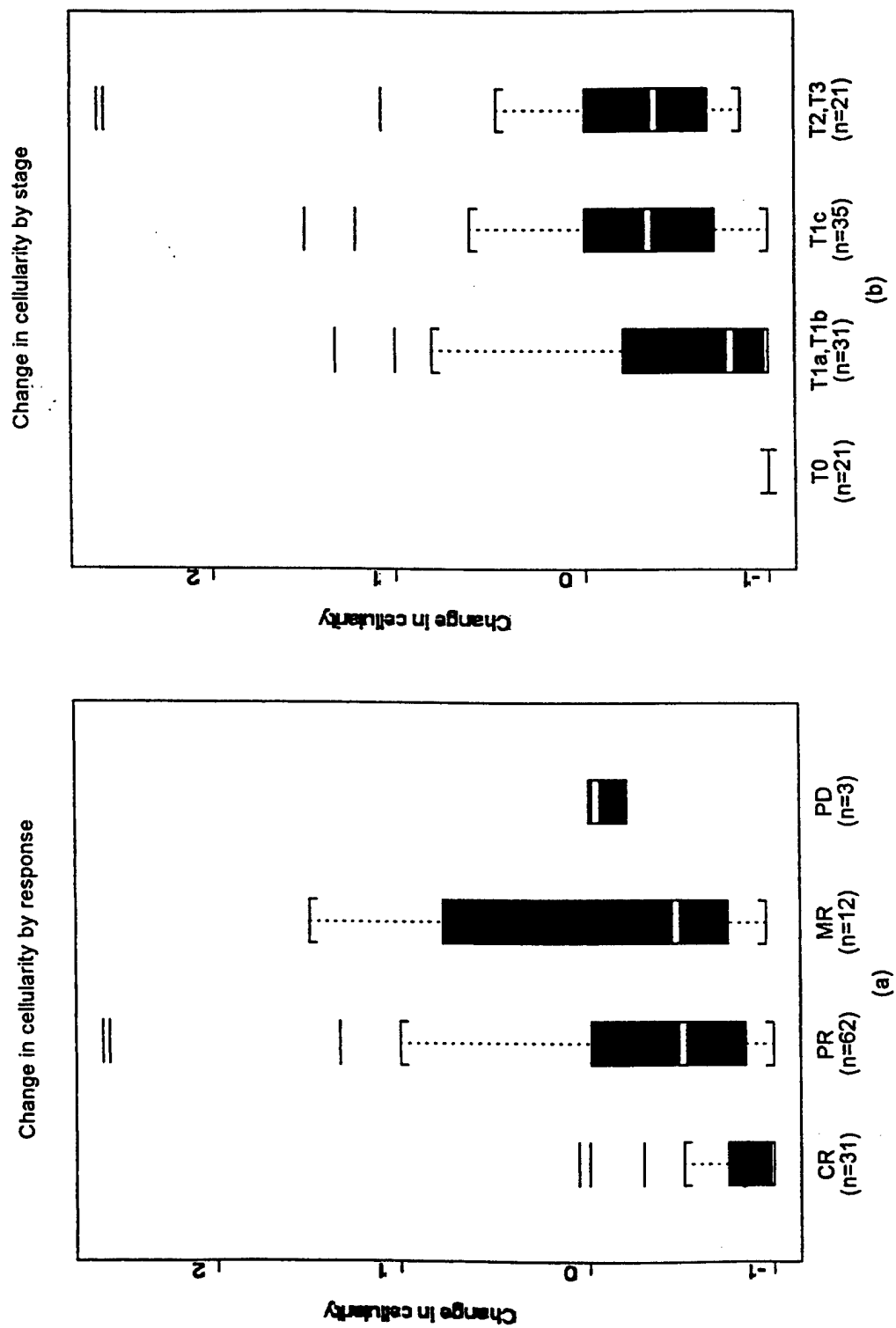


Figure 3 Change of cellularity by schedule

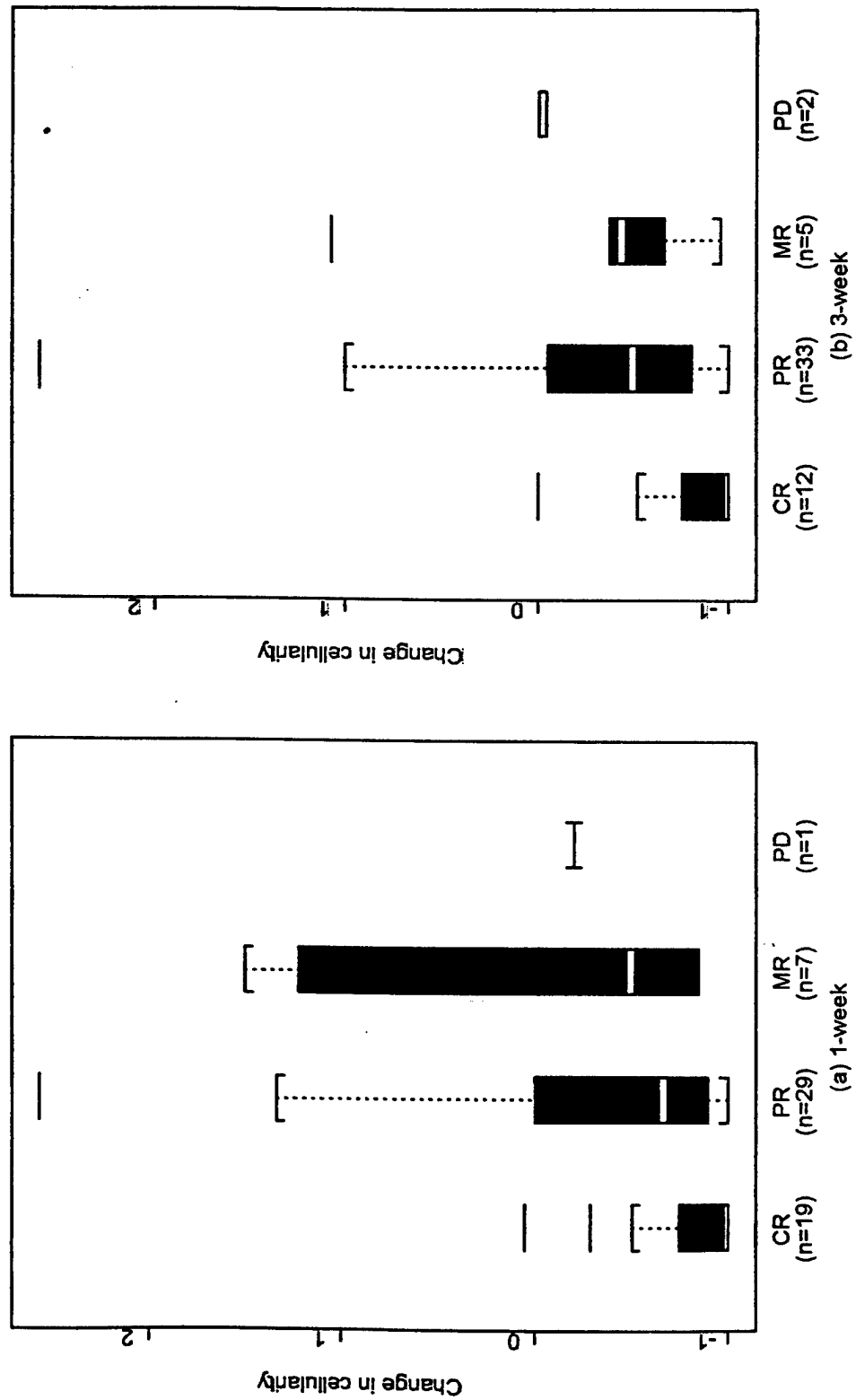


Figure 4.

